Full Paper

Synthesis, Anti-inflammatory and Analgesic Evaluation of Certain New 3*a*,4,9,9*a*-Tetrahydro-4,9-benzenobenz[*f*]isoindole-1,3-diones

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In an effort to establish new candidates with improved analgesic and anti-inflammatory activities, we reported here the synthesis and *in-vivo* analgesic and anti-inflammatory evaluation of various series of 2-substituted-3*a*,4,9,9*a*-tetrahydro-4,9-benzeno-benz[*f*]isoindole-1,3-diones: [4-Bromobutoxy] **6**, 5-bromopentoxy **7**, [4-(4-phenylpiperazin-1-yl)butoxy] **9**, [5-(4-phenylpiperazin-1-yl)pentoxy] **10**, 2-(2(4)-(4-phenylpiperazin-1-yl)-2-oxoethyl/4-oxobutyl **17**, **19**, [2(4)-(4-methylpiperazin-1-yl]-2-oxoethyl/4-oxobutyl **20**, **22**, [2(4)-morpholino-2-oxoethyl/4-oxobutyl] **23**, **25**, and 2(4)-(piperidin-1-yl)2-oxoethyl/4-oxobutyl) **26** and **28**. The newly synthesized compounds were characterized by (IR, ^{1}H-, ^{13}C-NMR, and mass spectra). The representative compounds were evaluated as analgesic and anti-inflammatory activities. Compounds **9**, **19**, **22**, **25**, and **28** exhibited activities higher than the reference drug.

Keywords: Analgesic and Anti-inflammatory Activities / Dibenzobarallene / Isoindoles / Secondary amines

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation in arthritis for decades. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity.

Therefore, the discovery of new safer anti-inflammatory drugs represents a challenging goal for such a research area [1–4]. It is evident from the literature that imide moiety is an integral part of structures of various important molecules such as fumaramidmycin [5, 6] granulatimide [7], isogranulatimide [8], rebeccamycin [9], and thalidomide [10, 11]. These molecules are reported to exhibit wide variety of biological activities such as antitumor [12, 13], anti-inflammatory [14], and antimicrobial [15]. Furthermore, *N*(-ω-aminoalkyl)imide **1** is an important pharmacolophore for central nervous system (CNS) depressant activity. Samant and Kulkarni have been reported the preparation and CNS depressant activity of a series of *N*-[(4-aryl-1-piprazinyl)alkyl]imides **2** [16–20]. They have been found that the chain length of the alkylene bridge and the nature of the imide part were crucial for the activity (Fig. 1). On the other hand, *N*-pyridinyl(alkyl)-phthalimides and *N*(-ω-aminoalkyl)-3*a*,4,9,9*a*-tetrahydro-4,9-benzenobenz[*f*]isoindole-1,3-diones were reported to possess anti-inflammatory [11, 21]. Furthermore, 4-alkoxy-2-[2-hydroxy-3-(4-aryl-1-piperazinyl) propyl]-6-methyl-1*H*-pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-diones display analgesic activity [22]. These biological data prompted us to synthesize some new *N*(-ω-aminoalkyl)-3*a*,4,9,9*a*-tetrahydro-4,9-benzeno-benz[*f*]isoindole-1,3-diones starting from dibenzobarallene [23], in order to investigate their anti-inflammatory and analgesic activity and to examine the effect of chain length of the alkylene bridge and amines on the activity.

Results and discussion

Chemistry

The target compounds were synthesized as outlined in Schemes 1–3. Starting compound **3** reacted with hydroxylamine as reported by Saikia *et al*. [24] to give **4**. Subsequent reaction of **4** with 1,3-dibromopropane, 1,4-dibromobutane or 1,5-dibromopentane furnished the corresponding cyclic imides **5–7**, respectively (Scheme 1). The structures **5–7** were confirmed on the basis of analytical and spectral data. The ^{1}H-NMR spectrum of **6** displayed multiplet signals

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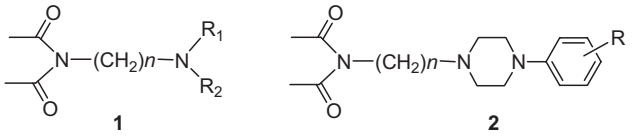
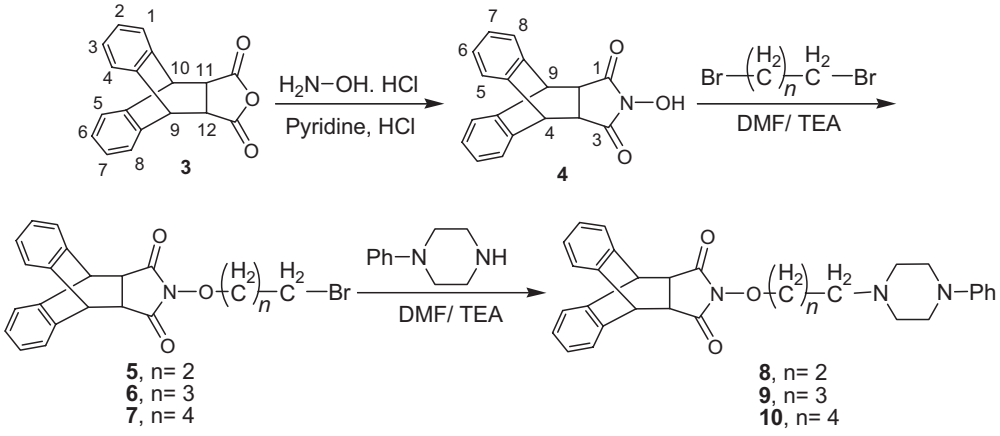


Figure 1. Structure of *N*(-ω-aminoalkyl)imide **1** and *N*-[(4-aryl-1-piprazinyl)-alkyl]imides **2** moieties.

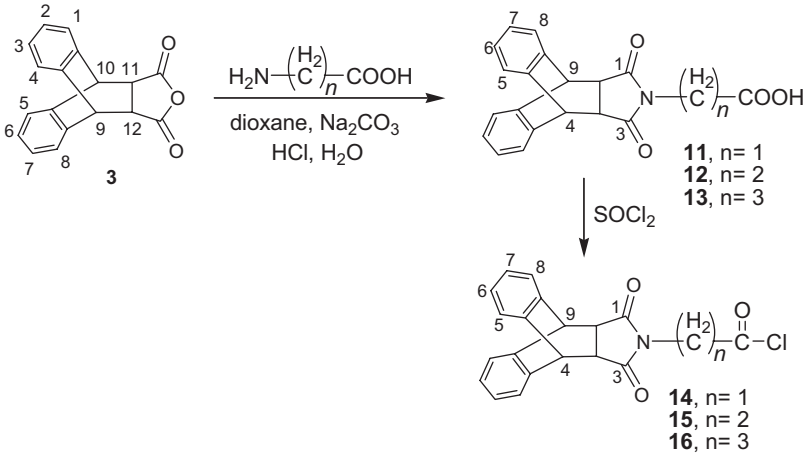
at δ 1.45-1.55, 1.86–1.88, and 3.36–3.38 ppm corresponding to (CH_2–CH_2–CH_2–CH_2–) protons. Furthermore, the ^{1}H-NMR of **7** revealed multiplet signals at δ 1.37–1.39, 1.55–1.56, 1.77–1.78, 3.12–3.14, and 3.36–3.37 due to (CH_2–CH_2–CH_2–CH_2–CH_2) protons. Treatment of **5**, **6**, or **7** with *N*-phenylpiperazine in DMF afforded the corresponding 3-(4-phenylpiperazin-1-yl)propyl derivative **8**, 4-(4-phenylpiperazin-1-yl)butyl derivative **9**, and 5-(4-phenylpiperazin-1-yl)pentyl derivative **10**, respectively. The presence of piperazine ring in **9** was confirmed by the ^{1}H-NMR spectrum, in which multiplet signals appeared at δ 2.57–2.58 and 3.12–3.18 ppm, also, compound **10** displayed multiple signals at δ 1.26–1.27, 1.33–1.34, 2.59–2.61, and 3.12–3.21 ppm corresponding to 18 protons of nine methylene groups. The ^{13}C-NMR spectrum of **9** revealed characteristic signals at δ 76.9, 49.2, 25.8, and 22.2 due to butyl carbons.

An attempt was also made to introduce a 2-oxoethyl, 3-oxypropyl or 4-oxobuyl linker between the nitrogen atoms of the imides group and piperazine ring, respectively. Therefore, the 2-ethanoic, 3-propanoic, and 4-butanoic acid derivatives **11–13** were prepared according to the previously reported method [25].

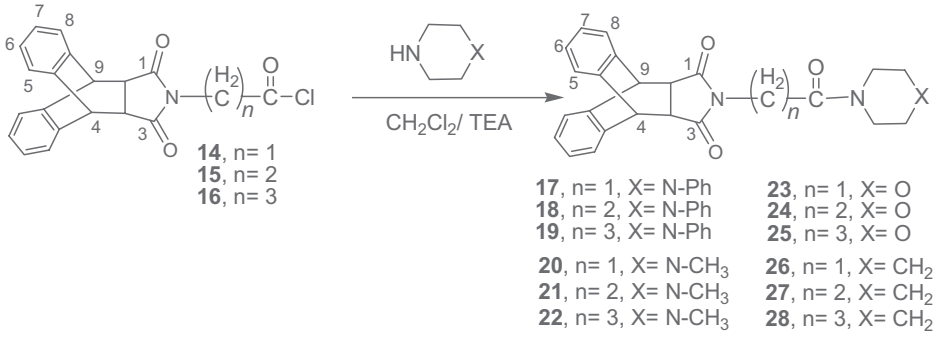
Reaction of **11–13** with thionyl chloride gave the acyl chloride derivatives **14–16** which on treatment with *N*-phenylpiperazine or *N*-methylpiperazine in methylene chloride containing a catalytic amount of triethylamine gave the corresponding amides **17–19** or **20–22**, respectively.



Scheme 1. Route for synthesis of benzeno-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione derivatives **8–10**.



Scheme 2. Synthesis of acid chloride derivatives **14–16**.



Scheme 3. Synthesis of 2-(substituted)-3*a*,4,9,9*a*-tetrahydro-4,9-[1,2]benzeno-1*H*-benzo[*f*]isoindole-1,3(2*H*)-dione derivatives **17–28**.

The ^{1}H-NMR spectrum of **17** and **19** displayed multiplet signals overlapping in the range at δ (3.35–3.44, 3.66–3.70) and (3.22–3.40, 3.55–3.80 ppm) due to eight protons of the piperazine ring respectively. Furthermore, **20** and **22** revealed singlet signals at δ 2.27 and 2.28 ppm, respectively, due to protons of methyl group. The ^{13}C-NMR spectrum of compound **17** exhibited signals at δ 39.1, 45.5, 47.3, 49.3, 49.5, 34.2, 41.2, 45.0, 46.6, and 167.9 ppm characteristic for piperazine and CH_2CO carbon atoms, respectively. Also, the ^{13}C-NMR spectrum of **22** revealed signals at δ 39.9, 37.8, 45.3, 45.9, 64.9, 54.6, 54.9, and 169.9 ppm characteristic for $N(CH_2)_2$ of piperazine and (CH_2CH_2–CH_2–CO) carbons, respectively, and an additional signal at δ 22.8 characteristic for methyl group.

Similarly, imides **23–25** and **26–28** were obtained by the reaction of **14**, **15** or **16** with morpholine and piperidine, respectively. The ^{1}H-NMR spectrum of **23** displayed multiplet overlapping in the range of δ 3.28–3.34 and δ 3.61–3.62 ppm due to eight protons of the morpholine ring. The ^{1}H-NMR spectrum of **25** revealed signals of analogous moiety at δ 3.18–3.19 and δ 3.68–3.69 ppm. Furthermore, compound **26** displayed multiplets overlapping in the range of δ 1.49–1.65 and δ 3.20–3.45 ppm due to eight protons of the piperidine ring. Moreover, the ^{1}H-NMR spectrum of **28** revealed signals of analogous moiety at δ 1.75–2.04 and δ 3.16–3.26 ppm. The ^{13}C-NMR spectrum of **23** revealed signals at δ 39.0, 42.4, 45.5, 47.2, 66.2, 66.7, and 163.3 ppm characteristic for morpholine and CH_2CO carbons, respectively, furthermore the piperidine and (CH_2CH_2–CH_2–CO) carbon atoms of **28** appeared at 22.9, 24.5, 25.5, 26.3, 30.1, 38.0, 46.3, 46.7, and 168.0 ppm, respectively.

Biological activity

Anti-inflammatory activity

Anti-inflammatory activity of the synthesized compounds was evaluated by carrageenan-induced paw edema test in rats. The data in Table 1 showed that compounds **9**, **19**, **22**, and **25** are more potent than Diclofenac sodium, while the rest of compounds showed moderate activities. On the other hand, all the tested compounds are more potent than the starting compound **3** (Table 1).

Analgesic Activity

The analgesic activity was determined by hot plate test (central analgesic activity) and acetic acid induced writhing assay. The results (Tables 2 and 3) revealed that all tested compounds exhibited significant activity. Compound **9**, **19**, **22**, **25**, and **28** exhibited activities higher than the reference drug. Furthermore, all synthesized compounds were more potent than the starting material **3**.

Table 1. Percent anti-inflammatory activity of the tested compounds (carrageenan-induced paw edema test in rats)

Compd. No.	Percent protection		
	1 hour	2 hours	3 hours
3	39.8 ± 1.42*	42.3 ± 1.42*	25.3 ± 1.32*
6	49.1 ± 1.28*	53.4 ± 1.40**	42.0 ± 1.35*
7	46.6 ± 1.55*	48.7 ± 1.41*	38.7 ± 1.42*
8	42.2 ± 1.39*	42.6 ± 1.48*	31.2 ± 1.34*
9	60.2 ± 1.82**	60.1 ± 1.55*	45.3 ± 1.60*
10	46.5 ± 2.27*	44.3 ± 1.75*	39.4 ± 1.10*
17	49.7 ± 2.44*	57.2 ± 1.65*	44.9 ± 1.84*
18	42.4 ± 1.65*	45.9 ± 1.47*	42.9 ± 1.63*
19	57.3 ± 1.38**	61.0 ± 1.68**	41.5 ± 1.08*
20	46.4 ± 1.55*	48.7 ± 1.42*	38.7 ± 1.40*
21	40.6 ± 1.44*	40.3 ± 1.44*	28.5 ± 1.53*
22	59.4 ± 1.05**	62.3 ± 2.02**	46.4 ± 1.25*
23	46.8 ± 2.29*	44.3 ± 1.84*	39.4 ± 1.06*
25	59.6 ± 1.40**	59.2 ± 1.30*	42.1 ± 1.24*
26	42.4 ± 1.65*	45.9 ± 1.48*	33.1 ± 1.27*
27	43.20 ± 1.61*	53.0 ± 1.97**	42.0 ± 1.94*
28	50.1 ± 1.51**	56.2 ±1.64**	32.4 ± 1.21*
29	44.1 ± 1.83*	49.0 ± 1.14	30.5 ± 1.83*
Control	6.2 ± 0.28	5.8 ± 0.43	3.3 ± 0.95
Diclofenac sodium	52.5 ± 0.94*	60.4 ± 1.54**	42.1 ± 1.38*

Each value represents the mean ± SE ($n = 6$).Significance levels $^{*}p < 0.5$, $^{**}p < 0.001$ as compared with respective control
Dose (20 mg/kg). For the selected tested compound

Table 2. Central analgesic activity (Hot plate test)

Group	Reaction time (min)			
	0 min	30 min	60 min	90 min
3	5.50 ± 0.58	6.50 ± 0.40	9.01 ± 0.70	10.01 ± 0.22 a
6	8.10 ± 0.52	9.80 ± 0.25 a	11.60 ± 0.55 a	11.80 ± 0.45 a
7	7.01 ± 0.10	8.55 ± 0.80	10.90 ± 0.90 a	11.10 ± 0.70 a b
8	5.70 ± 0.60	6.60 ± 0.49	9.05 ± 0.78	10.05 ± 0.25a
9	9.10 ± 0.30	9.22 ± 0.42a	10.35 ± 0.25 a	11.55 ± 0.55a b
10	6.55 ± 0.09	7.50 ± 0.25 b	8.58 ± 0.56 a	11.90 ± 0.32 a
17	8.05 ± 0.30	8.80 ± 0.30a	9.86 ± 0.55a	10.45 ± 0.45 b
18	5.90 ± 0.75	6.80 ± 0.60	9.48 ± 0.90	10.40 ± 0.29 a
19	8.40 ± 0.60	8.60 ± 0.32	9.05 ± 0.55 b	9.22 ± 0.46 b
20	7.20 ± 0.30	8.40 ± 0.40	9.80 ± 0.22	10.40 ± 0.12 b
21	6.09 ± 0.92	7.95 ± 0.75	10.18 ± 1.25	11.55 ± 0.42 a
22	8.92 ± 0.66	9.18 ± 0.58 a	10.79 ± 0.46 a	11.09 ± 0.25b
23	7.35 ± 0.35	9.38 ± 0.40 a	10.20 ± 0.25 a	11.60 ± 0.50 a b
25	9.05 ± 0.28	10.10 ± 0.25 b	10.70 ± 0.23 b	7.62 ± 0.52b
26	5.75 ± 0.65	6.70 ± 0.50	9.09 ± 0.80	10.08 ± 0.26 a
27	6.10 ± 0.98	7.99 ± 0.79	10.25 ± 1.25	11.60 ± 0.45 a
28	8.25 ± 0.39	9.55 ± 0.45	9.60 ± 0.50	10.35 ± 0.15 b
29	6.20 ± 0.55	7.05 ± 0.75 a	9.50 ± 0.80 a	12.60 ± 0.60 a
Control	8.24 ± 0.34	8.20 ± 0.38 b	8.70 ± 0.50 b	9.60 ± 0.45 b
Diclofenac sodium	6.49 ± 0.40	10.03 ± 0.12 a	11.39 ± 0.53 a	13.15 ± 0.38 a

[a] $P < 0.05$: Statistically significant from Control. (Dunnett's test). [b] $P < 0.05$: Statistically significant from ASA. (Dunnett's test)* Significant at $P < 0.0$. Values represent the mean ± SE of six animals for each group.

Table 3. Percent analgesic activity (Peripheral, writhing test)

Compd. No.	Percent protection			
	30 min	1 hour	2 hours	3 hours
3	38.40 ± 1.40*	43 ± 1.20*	47.1 ± 1.75	30.10 ± 1.20*
6	61.2 ± 1.51**	68 ± 1.55**	73.3 ± 1.80**	47.3 ± 170*
7	46.8 ± 1.51*	52 ± 1.49**	59.2 ± 1.69	65.2 ± 1.31*
8	39.40 ± 1.55*	44 ± 1.25*	48.2 ± 1.85	30.72 ± 1.25*
9	72.5 ± 1.01**	75.3 ± 1.35**	76.6 ± 1.30	62.3 ± 1.35**
10	45.0 ± 1.90*	53 ± 1.40*	55.6 ± 1.38	36.3 ± 1.20*
17	60.8 ± 1.15*	64.1 ± 1.30**	68.2 ± 1.03**	49.8 ± 1.70*
18	41.0 ± 1.60*	45 ± 1.30*	49.5 ± 1.90	33.2 ± 1.33*
19	62.4 ± 1.15*	64.3 ± 1.31**	68.4 ± 1.03**	50.2 ± 1.73*
20	50.5 ± 1.34*	52 ± 1.02*	55.4 ± 1.37	37.5 ± 1.49*
21	41.6 ± 1.16*	47 ± 1.32*	48.6 ± 1.36	36.4 ± 1,75*
22	68.0 ± 1.05**	71 ± 1.90**	72.4 ± 1.50**	57.2 ± 1.16*
23	60.2 ± 1.51**	68 ± 1.55**	73.3 ± 1.82**	46.3 ± 1.70*
25	69.0 ± 1.05**	72 ± 1.90**	74.4 ± 1.50**	57.4 ± 1.17*
26	40.5 ± 1.60*	45 ± 1.30*	49.2 ± 1.90	32.8 ± 1.30*
27	42.5 ± 1.44*	53 ± 1.27*	49.5 ± 1.94	37.8± 1.39*
28	62.1 ± 1.15*	64.3 ± 1.31**	68.4 ± 1.03**	50.3 ± 1.72*
29	44.5 ± 1.90*	48 ± 1.35*	52.1 ± 1.15	38.2 ± 1.80*
Control	02.0 ± 0.35	05.0 ± 0.50	04.0 ± 0.58	04.0 ± 0.90
Diclofenac sodium	45.0 ± 095*	54.2 ± 1.16*	61 ± 1.49*	38 ± 1.13*

Each value represents the mean ± SE ($n = 6$).Significance levels $^{*}p < 0.5$, $^{**}p < 0.001$ as compared with respective control. Dose (20 mg/kg). For the selected tested compound.

By comparing the results obtained from anti-inflammatory and analgesic activities of the tested compounds to their structures, the following structure activity relationships (SAR's) were postulated: (i) All imide derivatives are more potent than furandione derivative **3** which may attributable to the replacement of furandione moiety by pyrrolodinone. (ii) Compound **6** is more potent than **7** and compound **9** is more potent than **8** and **10** which may attributable to presence of propoxy moiety. (iii) Compounds **19**, **22**, **25**, and **28** are more potent than (**17**, **19**), (**20**, **21**) (**23**, **24**), and (**26**, **27**), respectively, which may attributable to presence of butanone moiety. (iv) Compound **22** is more potent than compounds **19**, **25**, and **28** which may attributable to the presence of *N*-methyl piperazine moiety (Fig. 2).

Conclusion

The prepared new ring systems seem to be interesting for biological activity studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of new isoindoles. It is worth mentioning that the chain length of the alkylene bridge at the 2-position of the isoindole was crucial for the anti-inflammatory activity, also, the butylene chain was ideal for the anti-inflammatory and analgesic activities as in the case of compounds **9**, **19**, **22**, **25**, and **28**.

Experimental

All melting points are in degree centigrade were determined on Gallenkamp electric melting point apparatus. The IR spectra were recorded on a Perkin Elmer Infrared Spectrophotometer Model 157 (Mansoura University, Faculty of Science, Mansoura, Egypt). The ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL-ECA500 (National Research Center, Giza, Egypt) and chemical shifts were expressed as δ values against TMS as internal standards. The mass spectra were recorded on GCMS-QP 1000 EX Shimadzu Japan (Gas Chromatography-Mass spectrometer). The microanalytical data were performed by the Microanalytical Center at National Research Center, Egypt. Biological activities were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt. Compounds **5**, **8**, **15**, **18**, **21**, **24**, and **27** were prepared according to the previously reported procedures [26].

*[2-(4-Bromobutoxy) and (5-bromopentoxy)]-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione **6** and **7***

General procedure

Compound **4** (5.83 g, 0.02 mol) was added portion-wise to a mixture of the corresponding halide, namely 1,4-dibromobutane (5.4 g, 0.025 mol) or 1,5-dibromopentane (5.74 g, 0.025 mol), and triethylamine (2.51 mL, 0.018 mol), in DMF (20 mL) and the reaction mixture was refluxed at 85–95°C for 3–5 h. The reaction mixture was poured into ice water (25 mL), the precipitated solid was filtered off, dried and recrystallized from benzene/ethanol (20 mL, ϕr = 3:1 for **6** and ϕr = 2:1 for **7**) to give pure products **6** and **7**, respectively.

Compound **6**: White crystals, yield, 83%, mp: 170°C, IR (KBr): ν_{max}, cm^{-1}: 2963 (aliphatic C–H), 1771, 1718 (2 C=O), 1221 (N–O), 662 (C–Br). ^{1}H-NMR ($CDCl_3$): δ 1.45–1.55 (m, 2H, CH_2), 1.86–1.88 (m, 2H, CH_2), 3.12–3.17 (m, 4H, H-11, H-12, CH_2-Br), 3.36–3.38 (m, 2H, CH_2O), 4.8 (s, 2H, H-9, H-10), 7.18–7.25 (m, 8H, H, aryl). MS: *m*/*z* (%) = 427 (M^+ + 1, 0.8), 425 (M^+, 0.8), 333 (0.5), 284 (0.8), 252 (2.4), 226 (1.5), 204 (0.4), 202 (0.4), 178 (100), 135 (6.5), 80 (22.1). Anal. calcd. for $C_{22}H_{20}BrNO_3$ (426.3): C, 61.98; H, 4.73; N, 3.29%. Found: C, 62.06; H, 4.79; N, 3.33%.

Compound **7**; White crystals, yield, 76%, mp: 128–130°C, IR (KBr): ν_{max}, cm^{-1}: 2963 (aliphatic C–H), 1771, 1718 (2 C=O), 1221 (N–O), 662 (C–Br). ^{1}H-NMR ($CDCl_3$): δ 1.37–1.39, (m, 2H, CH_2), 1.55–1.56 (m, 2H, CH_2), 1.77–1.78 (m, 2H, CH_2), 3.12–3.14 (m, 4H, H-11,

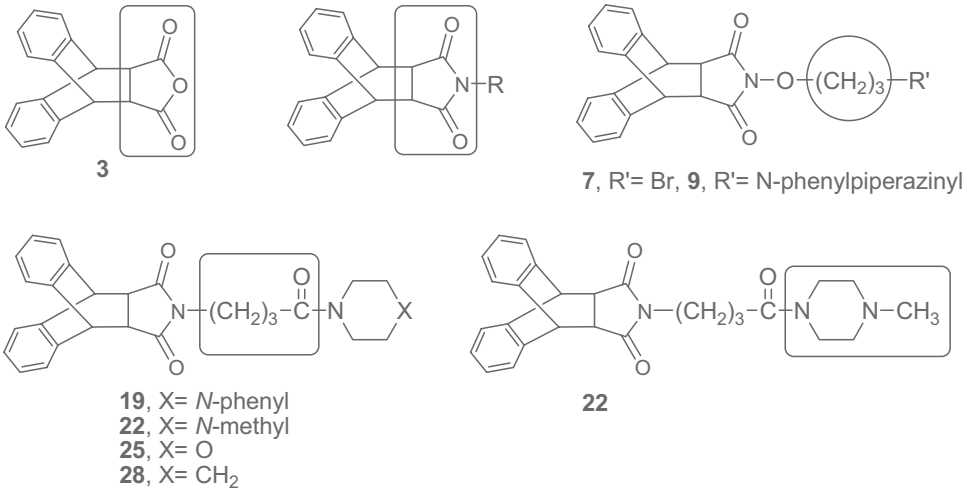


Figure 2. Structure activity relationships (SAR's) of the investigated compounds.

H-12, CH_2-Br), 3.36–3.37 (m, 2H, CH_2O), 4.80 (s, 2H, H-9, H-10), 7.16–7.38 (m, 8H, H, aryl). MS: m/z (%) = 441 (M^+ + 1, 3.4), 439 (M^+, 3.4), 298 (2.9), 372 (2.9), 308 (3.9), 263 (4.3), 250 (4.2), 204 (5.5), 202 (3.3), 186 (8.1), 178 (3.9), 82 (8.3), 75 (100), 55 (19.7). Anal. calcd. for $C_{23}H_{22}BrNO_3$ (440.33): C, 62.74; H, 5.04; N, 3.18%. Found: C, 62.78; H, 5.07; N, 3.24%.

*[2-(4-(4-Phenylpiperazin-1-yl)butoxy)] and [2-(5-(4-phenylpiperazin-1-yl)pentoxy)]3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-diones **9** and **10***

General procedure

A mixture of **6** (2.13 g, 0.005 mol) or **7** (2.2 g, 0.005 mol), *N*-phenylpiperazine (0.81 g, 0.005 mol), and triethylamine (0.98 mL, 0.007 mol) in DMF (10 mL) was heated at 90°C for 2 h The reaction mixture was poured into ice water (20 mL) and the obtained solid was crystallized from benzene/ethanol mixture (3:1) and benzene (20 mL) to give **9** and **10**, respectively.

Compound **9**: White crystals, yield, 81%, mp: 144°C, IR (KBr): ν_{max}, cm^{-1}: 2939 (aliphatic C–H), 1772, 1722 (2 C=O), 1231 (N–O). ^{1}H-NMR ($CDCl_3$): δ 1.33–1.47 (m, 4H, CH_2), 2.34–2.35 (m, 2H, CH_2N), 2.57–2.58 (m, 4H, piprazine), 3.12–3.18 (m, 8H, H-11, H-12, OCH_2, piprazine), 4.81 (s, 2H, H-9, H-10), 6.85–7.25 (m, 13H, H, aryl). ^{13}C-NMR ($CDCl_3$): δ 171.6, 151.4, 140.7, 138.6, 129.2, 127.1, 125.3, 124.4, 119.8, 116.1, 57.9, 53.3, 49.2, 45.4, 43.7, 25.8, 22.2. MS: m/z (%) = 504 (M^+ − 3 H, 2.4), 498 (4.3), 415 (3.9), 384 (3.1), 316 (6.8), 265 (2.4), 242 (7.7), 226 (11.6), 178 (58.0), 95 (15.2), 74 (100), 58 (95.5). Anal. calcd. for $C_{32}H_{33}N_3O_3$ (507.62): C, 75.71; H, 6.55; N, 8.28%. Found: C, 75.65; H, 6.51; N, 8.23%.

Compound **10**: White crystals, yield, 79%, mp: 128–130°C, IR (KBr): ν_{max}, cm^{-1}: 2939 (aliphatic C–H), 1772, 1722 (2 C=O), 1231 (N–O). ^{1}H-NMR ($CDCl_3$): δ 1.26–1.27 (m, 2H, CH_2), 1.33–1.34 (m, 2H, CH_2), 2.34–2.35 (m, 2H, CH_2N), 2.59–2.61 (m, 4H, piprazine), 3.12–3.21 (m, 8H, H-11, H-12, OCH_2, piprazine), 4.81 (s, 2H, H-9, H-10), 6.83–7.39 (m, 13H, H, aryl). MS: m/z (%) = 521 (M^+, 0.05), 414 (2.7), 361 (2.4), 218 (2.0), 126 (1.8), 78 (92.7), 63 (100), 45 (81.8). Anal. calcd. for $C_{33}H_{35}N_3O_3$ (521.65): C, 75.98; H, 6.76; N, 8.06%. Found: C, 76.07; H, 6.84; N, 8.16%.

*2-(1,3-Dioxo-3a,4-dihydro-4,9-[1,2]benzeno-1H-benzo[f]isoindol-2(3H,9H,9aH)-yl)ethanoylchloride (**14**) and 4-(1,3-dioxo-3a,4-dihydro-4,9-[1,2]benzeno-1H-benzo[f]isoindol-2(3H,9H,9aH)-yl)butanoylchloride (**16**)*

General procedure

Compound **11** (13.33 g, 0.04 mol) or **13** (14.46 g, 0.04 mol) was treated with thionyl chloride (75 mL, 1.03 mol) and the reaction mixture was refluxed for 8 h. The excess of thionyl chloride was distilled off and the residue was crystallized from benzene (120 mL) to give **14** and **16**, respectively.

Compound **14**: White powder, yield, 91%, mp: 183–185°C, IR (KBr): ν_{max}, cm^{-1}: 2937 (aliphatic C–H), 1779, 1760, 1718 (2 C=O). Anal. calcd. for $C_{20}H_{14}ClNO_3$ (351.78): C, 68.28; H, 4.01; N, 3.98%. Found: C, 68.36; H, 4.11; N, 4.02%.

Compound **16**: White powder, yield, 93%, mp: 168–170°C, IR (KBr): ν_{max}, cm^{-1}: 2952 (aliphatic C–H), 1771, 1764, 1731 (2 C=O). Anal. calcd. for $C_{22}H_{18}ClNO_3$ (379.84): C, 69.57; H, 4.78; N, 3.69%. Found: C, 69.64; H, 4.87; N, 3.71%.

Reactions of 2(4)-[1,3-dioxo-3a,4-dihydro-4,9-[1,2]benzeno-1H-benzo[f]isoindol-2(3H,9H,9aH)-yl][(ethanoyl), (butanoyl)] chlorides (14), (16) with secondary amines

General procedure

A mixture of **14** (1.76 g, 0.005 mol) or **16** (1.899 g, 0.005 mol), triethylamine (0.56 mL, 0.004 mol), and the corresponding amines, namely *N*-phenylpiperazine (0.81 g, 0.005 mol), *N*-methylpiperazine (0.5 g, 0.005 mol), morpholine (0.44 g, 0.005 mol) or piperidine (0.43 g, 0.005 mol) (each amine was reacted with compounds **14** and **16**, respectively) was refluxed in dichloromethane (30 mL) for 2 h. The solvent was evaporated under reduced pressure and the residue was washed with aqueous ethanol (20 mL, 80 vol. %). The obtained solid was crystallized from the appropriate solvent to give **17**, **19**, **20**, **22**, **23**, **25**, **26**, and **28**, respectively.

*2-(Oxo-2-(4-phenylpiperazin-1-yl)-ethyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**17**)*

White powder, yield, 98%, mp: 252°C, crystallization from DMF/ethanol mixture (1:5), IR (KBr): ν_{max}, cm^{-1}: 2983, 2954, (aliphatic C–H), 1751, 1708, 1654 (3 C=O). ^{1}H-NMR ($CDCl_3$): δ 3.12 (s, 2H, H-11, H-12), 3.35–3.44 (m, 4H, $(CH_2)_2NPh$), 3.66–3.70 (m, 4H, $(CH_2)_2NCO$), 3.80 (s, 2H, $(CO)_2NCH_2$), 4.8 (s, 2H, H-9, H-10), 6.88–7.37 (m, 13H, H, aryl). ^{13}C-NMR ($CDCl_3$): δ 176.4, 163.2, 150.8, 141.5, 138.9, 129.4, 125.0, 124.4, 120.9, 116.9, 49.5, 49.3, 47.3, 45.5, 45.0, 44.7, 42.2, 41.2, 39.0. MS: m/z (%) = 478 (M^+ + 1, 1.5), 477 (M + 1, 1.8), 440 (1.8), 388 (1.7), 259 (1.9), 204 (4.3), 202 (3.0), 178 (32.3), 132 (18.9), 78 (89.4), 63 (100). Anal. calcd. for $C_{30}H_{27}N_3O_3$ (477.55): C, 75.45; H, 5.70; N, 8.80%. Found: C, 75.53; H, 5.76; N, 8.78%.

*2-(4-Oxo-4-(4-phenylpiperazin-1-yl)-butyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**19**)*

White crystals, yield, 93%, mp: 156°C, crystallization from benzene/ethanol mixture (2:1), IR (KBr): ν_{max}, cm^{-1}: 2829, (aliphatic C–H), 1751, 1704, 1635 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.72–1.83 (m, 2H, CH_2), 2.22–2.34 (m, 2H, CH_2), 3.16 (s, 2H, 2H, H-11, H-12), 3.22–3.40 (m, 4H, $(CH_2)_2NPh$), 3.55–3.80 (m, 6H, $(CH_2)_2NCO$, $(CO)_2NCH_2$), 4.85 (s, 2H, H-9, H-10), 6.83–7.42 (m, 13H, H, aryl). Anal. calcd. for $C_{32}H_{31}N_3O_3$ (505.61): C, 76.02; H, 6.18; N, 8.31%. Found: C, 76.11; H, 6.20; N, 8.37%.

*2-(2-Oxo-2-(4-methylpiperazin-1-yl)-ethyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**20**)*

White powder, yield, 88%, mp: 327°C, crystallization from DMF/ethanol mixture (1:4), IR (KBr): ν_{max}, cm^{-1}: 2983 (aliphatic C–H), 1751, 1706, 1652 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 2.27 (s, 3H, CH_3), 3.31–3.34 (m, 6H, H-11, H-12, $(CH_2)_2NCH_3$), 3.53–3.66 (m, 4H, CH_2CH_2NCO), 3.74 (s, 2H, CH_2), 4.78 (s, 2H, H-9, H-10), 7.10–7.37 (m, 8H, H, aryl). Anal. calcd. for $C_{25}H_{25}N_3O_3$ (415.48): C, 72.27; H, 6.06; N, 10.11%. Found: C, 72.35; H, 6.14; N, 10.17%.

*2-(4-Oxo-4-(4-methylpiperazin-1-yl)-butyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**22**)*

White crystals, yield, 89%, mp: 198°C, crystallization from benzene, IR (KBr): ν_{max}, cm^{-1}: 2933, 2804, (aliphatic C–H), 1749, 1704, 1635 (3 C=O). ^{1}H-NMR (DMSO-d_6): δ 1.72–1.74 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.31 (m, 2H, CH_2CO), 3.12–3.22 (m, 6H, H-11, H-12, $(CH_2)_2NCH_3$), 3.43–3.56 (m, 4H, $(CH_2)_2NCO$), 3.70 (s, 2H, $CH_2N(CO)_2$), 4.74 (s, 2H, H-9, H-10), 7.07–7.31 (m, 8H, H, aryl). ^{13}C-NMR ($CDCl_3$): δ 176.7, 169.9, 141.3, 138.7, 126.8, 124.7, 124.1, 54.9, 54.6, 46.6, 45.9, 45.3, 45.0, 41.3, 37.8, 29.9, 22.8. Anal. calcd. for $C_{27}H_{29}N_3O_3$ (443.54): C, 73.11; H, 6.59; N, 9.47%. Found: C, 73.07; H, 6.52; N, 9.38%.

*2-(2-Oxo-2-(morpholin-1-yl)-ethyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**23**)*

White crystals, yield, 87%, mp: 299°C, crystallization from ethanol/benzene mixture, IR (KBr): ν_{max}, cm^{-1}: 2900, 2848 (aliphatic C–H), 1749, 1706, 1652 (3 C=O). ^{1}H-NMR ($CDCl_3$): δ 3.28–3.34 (m, 6H, H-11, H-12, $N(CH_2)_2$), 3.61–3.62 (m, 4H, $O(CH_2)_2$), 3.73 (s, 2H, $(CO)_2NCH_2$), 4.79 (s, 2H, H-9, H-10), 7.11–7.35 (m, 8H, H, aryl). ^{13}C-NMR ($CDCl_3$): δ 176.3, 163.3, 141.5, 138.9, 127.0, 126.9, 125.0, 124.4, 66.7, 66.2, 47.2, 45.5, 45.1, 42.4, 39.0. Anal. calcd. for $C_{24}H_{22}N_2O_4$ (402.44): C, 71.63; H, 5.51; N, 6.96%. Found: C, 71.70; H, 5.56; N, 7.04%.

*2-(4-Oxo-4-(morpholin-1-yl)-butyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**25**)*

White powder, yield, 69%, mp: 220°C, crystallization from ethanol/benzene mixture, IR (KBr): ν_{max}, cm^{-1}: 2852 (aliphatic C–H), 1751, 1702, 1641 (3 C=O). ^{1}H-NMR ($CDCl_3$): δ 1.60–1.75 (m, 2H, CH_2), 1.70–1.78 (m, 2H, CH_2), 3.17 (s, 2H, H-11, H-12), 3.18–3.19 (m, 4H, $N(CH_2)_2$), 3.65–3.66 (m, 2H, $(CO)_2NCH_2$), 3.68–3.69 (m, 4H, $O(CH_2)_2$), 4.78 (s, 2H, H-9, H-10), 7.11–7.27 (m, 8H, H, aryl). Anal. calcd. for $C_{26}H_{26}N_2O_4$ (430.5): C, 72.54; H, 6.09; N, 6.51%. Found: C, 72.60; H, 6.11; N, 6.54%.

*2-(2-Oxo-2-(piperidin-1-yl)ethyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**26**)*

White crystals, yield, 82%, mp: 322°C, crystallization from DMF/ethanol mixture (1:3), IR (KBr): ν_{max}, cm^{-1}: 2985 (aliphatic C–H), 1749, 1708, 1652 (3 C=O). ^{1}H-NMR ($CDCl_3$): δ 1.49–1.65 (m, 6H, piperidine), 3.20–3.45 (m, 6H, H-11, H-12, $N(CH_2)_2$), 3.73 (s, 2H, $(CO)_2NCH_2$), 4.79 (s, 2H, H-9, H-10), 7.11–7.25 (m, 8H, H, aryl). MS: m/z (%) = 400 (M^+, 4.5), 296 (3.6), 270 (50), 242 (5.4), 204 (3.6), 202 (5.4), 178 (17.2), 167 (5.5), 135 (4.5), 63 (100), 45 (90.9). Anal. calcd. for $C_{25}H_{24}N_2O_3$ (400.47): C, 74.98; H, 6.04; N, 7.00%. Found: C, 74.91; H, 6.01; N, 6.96%.

*2-(4-Oxo-4-(piperidin-1-yl)butyl)-3a,4,9,9a-tetrahydro-4,9-[1,2]benzeno-1H-benzo[f]isoindole-1,3(2H)-dione (**28**)*

White crystals, yield, 90%, mp: 175°C, crystallization from ethanol, IR (KBr): ν_{max}, cm^{-1}: 2940, 2952 (aliphatic C–H), 1749, 1700, 1635 (3 C=O). ^{1}H-NMR ($CDCl_3$): δ 1.75–2.04 (m, 8H, CH_2, 3 CH_2, piperidine), 2.14 (m, 2H, CH_2–CO), 3.16–3.26 (m, 6H, H-11, H-12, $N(CH_2)_2$), 3.49–3.52 (m, 2H, $(CO)_2NCH_2$), 4.77 (s, 2H, H-9, H-10), 7.10–7.34 (m, 8H, H, aryl). ^{13}C-NMR ($CDCl_3$): δ 176.8, 168.0, 141.5, 138.8, 126.9, 124.9, 124.2, 46.7, 46.3, 45.4, 42.6, 38.0, 30.1, 26.3, 25.5, 24.5, 22.9. MS: m/z (%) = 428 (M^+, 0.9), 355 (100), 336 (10.5), 326 (8.8), 300 (6.9), 264 (70.5), 209 (5.8), 178 (2.3), 142 (3.5), 119 (6.4), 84 (1.5), 81 (5.2), 62 (9.4). Anal. calcd. for $C_{27}H_{28}N_2O_3$ (428.52): C, 75.68; H, 6.59; N, 6.54%. Found: C, 75.74; H, 6.63; N, 6.57%.

Biological activity

Material and methods

Female Sprague-Dawley rats (150–200 g) were used in the study of anti-inflammatory activity. Both sex of Swiss mice weighing (25–30 g) used in analgesic activity and, taking into account international principle and local regulations concerning the care and used of laboratory animals [27]. The animals had free access to standard commercial diet and water *ad libitum* and were kept in rooms maintained at 22 ± 1°C with 12 h light dark cycle.

Anti-inflammatory activity (carrageenan-induced rat hind paw edema model)

The method adopted, described by Winter *et al*. [28] (distilled water), was selected as vehicle to suspend the standard drugs and the test compounds. The albino rats weighing between 150–180 g were starved for 18 h prior to the experiment. The animals were weighed, marked for identification, and divided into 20 groups each group containing 6 animals. Edema was induced in the left hind paw of all rats by subcutaneous injection of 0.1 mL of 1% (w/v) carrageenan in distilled water into their footpads. The 1st group was kept as control and was given the respective volume of the solvent (0.5 mL distilled water). The 2nd to 19th group was orally administered aqueous suspension of the synthesized compounds in dose of (20 mg/kg) 1 h before carrageenan injection. The last group (standard) was administered Diclofenac sodium in a dose of 20 mg/kg, orally as aqueous suspension [29]. The paw volume of each rat was measured immediately by mercury plethysmometer, before carrageenan injection and then hourly for 4 h post administration of aqueous suspension of the synthesized compounds. The edema rate and inhibition rate of each group were calculated as follows:

$$\text{Edema rate } (E)\% = V_t - V_o / V_o$$
$$\text{Inhibition rate } (I)\% = E_c - E_t / E_c$$

where V_t is the volume before carrageenan injection (mL), V_t is the volume at t hours after carrageenan injection (mL) E_c, E_t are the edema rate of control and treated groups, respectively.

Analgesic activity using hot-plate test

The experiment was carried out as described by Turner [30], using hot-plate apparatus, maintained at 53 ± 0.5°C. The

mice were divided into 20 groups of 6 animals each. The reaction time of the mice to the thermal stimulus was the time interval between placing the animal in the hot plate and when it licked its hind paw or jumped. The reaction time was measured prior to aqueous suspension of synthesized compounds and drug treatment (0 min). Group 1 was kept as normal control. The aqueous suspension of synthesized compounds was orally administered to mice of groups 2 to 19 at doses of 20 mg/kg. Mice of group 20 (reference) were orally treated with Diclofenac sodium in a dose of 20 mg/kg body wt. The reaction time was again measured at 15 min. and repeated at 30, 60, and 90 min. after treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60 s. The reaction time was calculated for each synthesized compounds and drug-treated group.

Analgesic activity (acetic acid induced Writhing response model)

The compounds were selected for investigating their analgesic activity in acetic acid induced writhing response in Swiss albino mice, following the method of Collier *et al*. [31]. One hundred and twenty six mice were divided into 20 groups (six in each group) starved for 16 h pretreated as follows, the 1st group which served as control positive was orally received distilled water in appropriate volumes. The 2nd to 19th groups were received the aqueous suspension of synthesized compounds orally at dose (20 mg/kg). The last group was orally received Diclofenac sodium in a dose of 20 mg/kg. After 30 min, each mouse was administrated 0.7% of an aqueous solution of acetic acid (10 mL /kg) and the mice were then placed in transparent boxes for observation. The number of writhes was counted for 20 min after acetic acid injection. The number of writhes in each treated group was compared to that of a control group. The number of writhing was recorded and the percentage protection was calculated using the following ratio:

$$(\%)\,\text{protection} = (\text{control}_{\text{mean}} - \text{treated}_{\text{mean}}/\text{control}_{\text{mean}}) \times 100$$

The authors have declared no conflict of interest.

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